Geographic and Reproductive Isolation in the Marine Harpacticoid Copepod *Tisbe*

B. Battaglia and B. Volkmann-Rocco

Istituto di Biologia Animale, Università di Padova; Padua, Italy

and

Istituto di Biologia del Mare, C.N.R.; Venice, Italy

Abstract

Within the genus *Tisbe* (Copepoda:Harpacticoida), there is a wide range of reproductive isolation. In the European area, in addition to species where (between geographic populations) the reproductive barrier is practically absent, there are others which show a varying degree of incompatibility. The present paper deals with the results of cross-breeding experiments carried out between American and European populations. The respective intrapopulation crosses served as controls. Transatlantic crosses have been attempted with the following species: *T. lagunaris*, *T. bulbisetosa*, *T. holothuriae*, *T. battagliai*, and *T. clodiensis*. The American and European populations of the first 4 species are interfertile, and the F₁ hybrids produce viable offspring. Moreover, the interpopulation crosses, compared to the controls, exhibit a slight superiority whose nature is, however, still unclear. The fifth species, *T. clodiensis*, behaves differently. The absence of viable offspring from interpopulation crosses, or the death of the hybrids before reaching the adult stage, indicate that, in this species, the barrier to gene flow is complete. Populations of *T. clodiensis* show, in addition, signs of “relative intraspecific incompatibility”. The problem of the different degree of reproductive isolation in the genus *Tisbe*, with special regard to the role played by ecological factors in determining the isolation patterns, is discussed.

Introduction

The genus *Drosophila* has, so far, provided the best material for research on speciation. A possible marine counterpart is the harpacticoid copepod *Tisbe*. It is a good organism for genetic experimentation and, during the last few years, sufficient data, both observational and experimental, have become available on species divergence.

The stimulus for pursuing such research in *Tisbe* has been the taxonomic difficulties inherent in this genus (Volkmann-Rocco, 1971), partly due to the existence of morphologically very similar forms considered as sibling species (Volkmann-Rocco and Fava, 1969; Volkmann-Rocco and Battaglia, in press).

The genus *Tisbe* exhibits a wide range of reproductive isolation, which invites comparative study. In addition to species characterized by almost complete absence of reproductive barriers between geographically separate populations, there are other species that exhibit incipient reproductive isolation, and still further species where populations comparatively close to each other are reproductively incompatible. Thus, in the latter case, the process of species splitting has reached the critical stage of transition from race to species.

Our efforts to date have been confined to populations living in European waters (Battaglia, 1957; Battaglia and D'Avella, 1964; Battaglia and Volkmann-Rocco, 1969). Recently, populations of *Tisbe* from the Atlantic coast of the USA have become available, and a number of interpopulation transatlantic crosses have been attempted. The main results of these crosses are reported in the present paper.

Material and Methods

The material consists of 5 species of *Tisbe*, whose European populations were collected as follows: *T. lagunaris* Volkmann-Rocco (1972a) from Venice in summer, 1970; *T. bulbisetosa* Volkmann-Rocco (1972a) from the Lagoon of Venice in 1969; *T. holothuriae* Humes (1957) from Helgoland in 1969; *T. battagliai* Volkmann-Rocco (1972b) from Anzio in 1968; *T. clodiensis* Battaglia and Fava (1968) from the Lagoon of Venice in 1967 and from Banyuls-sur-Mer (France) in 1968. The American populations of the above species, used for the transatlantic crosses, were all collected in Beaufort, North Carolina, in May, June, and July, 1970.

The stocks utilized for the experiments come from populations raised in the laboratory for a period of 1 month to several years, employing the standard cultivation technique suggested by Battaglia (1970). All cross-breeding experiments were carried out at a constant temperature of 18 °C and at a salinity of about 35 to 36 ppt.

Twenty to 30 ovigerous females were taken at random from the laboratory cultures of the above populations, and isolated in single dishes. As soon as the nauplii hatched, the females were removed. When the 4th or 5th copepodite stage was reached, pairs were set up: one female copepodite and one male...
copepodite per dish. Each dish was checked twice a day to observe mating and to discard pairs of the same sex. As soon as the female became ovigerous, she was transferred to a freshly prepared dish; she was removed immediately after the hatching of the nauplii. The offspring had attained adulthood, they were counted and sexed. Two to 10% of the isolated pairs were generally discarded because of: (1) early death of one of the mates; (2) wrongly matched pairs; (3) egg sacs which did not produce viable offspring.

For *Tisbe lagunaris*, the first experiment was carried out 1 month after the Venice population and 3 months after the Beaufort population were set up. Because of the comparatively small number of pairs, a replicate was carried out after 7 months, except in the case of the cross ♀ Venice x ♂ Beaufort, where the number of pairs was sufficiently high. Due to lack of significant differences between replicates, the data have been pooled. The pairs for the experimental series as well as those for the control series were made 8 to 9 days after hatching of the nauplii.

For *Tisbe bulbisetosa*, at the initiation of the experiment, the Beaufort population had been kept in the laboratory for about 7 months, and the Venice population for several years. Pairs were set up 8 to 9 days after hatching of the nauplii.

The experiments with *Tisbe holothuriae* were carried out 1 month after the Beaufort specimens had been collected, whereas the European population (in this case from Helgoland) had been kept in the laboratory for a year. The crosses ♀ Helgoland x ♂ Beaufort and their controls were repeated 8 months later, as the previous data were considered insufficient. However, since the data of the two series of crosses were similar, they have been pooled. Pairs were set up 7 to 8 days after hatching of the nauplii.

Also, in the case of *Tisbe battagliai*, the interpopulation crosses and their respective controls were carried out twice: shortly after collection of the Beaufort specimens; and 8 months later. Again, it was possible to consider the two replicates as a single experiment. The pairs were made 6 to 7 days after hatching of the nauplii.

The experiments with *Tisbe clodiensis* were performed 8 months after the Beaufort specimens had been collected, whereas the European populations (Venice and Banyuls) had already been kept in the laboratory between 3 and 4 years. Pairs were set up 8 days after hatching of the nauplii.

**Results**

The results of the crosses carried out between *Tisbe* populations from Europe and the United States are summarized in Tables 1 and 2. In Table 1, the figures give the average number of F₁ adult individuals per female produced from the first egg sac only. The figures of the last column (viability) express the survival of the interpopulation hybrids to the adult stage, and give, therefore, a good measure of the degree of isolation. The viability of F₁ individuals in interpopulation crosses is taken to be unity, and the hybrids are characterized by ratios of their scores divided by the "control" values.

The main result emerging from the data reported in Table 1, which concern the interpopulation crosses carried out on *Tisbe lagunaris*, *T. bulbisetosa*, *T. holothuriae*, and *T. battagliai*, is that the European and American populations of these 4 species interbreed perfectly. This is also supported by the "viability" figures, which in no case are smaller in interpopulation than in intrapopulation crosses. There is an indication of a slight superiority of the former.

In previous interpopulation crosses carried out in another species, *Tisbe reticulata* (Battaglia, 1967), there were strong deviations in the sex-ratio favouring the males. This has been interpreted as an indication of incipient incompatibility. No such extremes in sex-ratio deviations were found in the interpopulation crosses for the species considered in the present paper.

It has been possible to detect a relationship between the number of F₁ individuals produced by each female (a value which not only gives a measure of survival but, in part, reflects also fecundity) and her geographic origin. In nearly all cases, differences in the number of offspring are smaller between the transatlantic crosses and their respective controls than between the reciprocal crosses or between the controls of different geographic origin.

Although our analysis has not been extended to the F₂ hybrids, we have nevertheless observed that, in all crosses considered so far, the F₁ hybrids are fertile and give viable offspring.

Completely different is the situation in *Tisbe clodiensis*. As might be expected from previous cross-breeding experiments between several Mediterranean populations (Battaglia and Volkmann-Rocco, 1969), there exists a strong reproductive barrier between European and American populations. The data are summarized in Table 2. The values refer to the number of matings in which (1) the females produced no egg sacs; (2) sacs were extruded but eggs did not develop into nauplii; (3) eggs reached the naupliar stage; (4) eggs reached the copepodite stage.

In the cross ♀ Venice x ♂ Beaufort, mating takes place regularly, and also hatching of the nauplii is normal, as in the controls. However, while in the control series the first F₁ adults already appeared after 11 days, in the interpopulation cross there was a high naupliar mortality, and it took 9 to 19 days just to reach the second or third copepodite stage. Moreover, the few copepodites which appeared in the F₁ generation died after 6 to 24 days. Nauplii as well as copepodites were morphologically abnormal.